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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 898 967 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
03.03.1999 Bulletin 1999/09

(51) Int. Cl.[6]: **A61K 35/34**, A61K 48/00

(21) Application number: **98201068.8**

(22) Date of filing: **07.04.1998**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **11.08.1997 US 55199 P**

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(54) Myoblast transfer therapy for relieving pain and for treating behavioral and perceptive abnormalities

(57) An analgesic benefit is realized by continuously supplying a peptide *in vivo* that activates an opioid receptor or that interferes with the binding of substance P to its receptors. The long-term, continuous provision of such a peptide can be accomplished by (a) transducing myogenic cells with DNA expressing the peptide and (b) administering the transduced myogenic cells to a patient, such that the cells continuously produce the peptide.

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Printed by Xerox (UK) Business Services
2.16.7/3.6

Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to an approach for relieving pain and for treating behavioral and perceptive abnormalities, by using myoblast transfer therapy to provide a long-term, continuous supply of peptides *in vivo* that have analgesic activity.

[0002] Modern analgesia theory advanced significantly with a proposal by Pomeranz *et al., Exp. Neurol.* **54**: 172 (1977), that a morphine-like, pituitary peptide mediates acupuncture analgesia. Electroacupuncture was found to reduce responses in spinal cord neurons to noxious stimuli in anesthetized cats and to increase squeak threshold in awake mice. The observed, prolonged time course implicated a hormonal mechanism for the response. Spinal transaction, decerebration or hypophysectomy eliminated this acupuncture effect, and intravenous injections of naloxone, a morphine antagonist, also reduced it markedly.

[0003] These results indicate that electroacupuncture stimulates sensory nerves which activate the pituitary glands to release morphine-like hormones (peptides) effecting prolonged reduction in transmission along nociceptive pathways. This mechanism is believed to be a principal mediator of generalized and localized analgesia.

[0004] Morphine-like peptides have been identified, and receptors to morphine-like peptides and to other opioid peptides have been found in the brain, the gut, the pituitary gland, the pancreas and the placenta. Hughes *et al., Nature* **258**: 577 (1975); Pert *et al., Science* **179**: 1011 (1979). These peptides now are known as β-endorphins and enkephalins. Cooper *et al.*, THE BIOCHEMICAL BASIS OF PHARMACOLOGY, 4th ed. (Oxford University Press, New York 1982). Furthermore, stimulation of brain neurons with an opioid peptide such as an endorphin produces analgesia. Fields *et al., Ann. Rev. Physiol.* **40**: 217 (1978). This effect can be reversed by naloxone.

[0005] Opioid peptides, especially β-endorphins, are essentially neural hormones or transmitters which reach all body tissue through diffusion. The presence of endorphin receptors in large numbers at different areas of the diencephalon and cerebral cortex suggests that the conjugate opioid peptides play a role in analgesia which goes beyond that of a simple modulator of pain perception. Covenas *et al. Neuropeptides* **30**: 261 (1996); Bernstein *et al., Neurosci. Lett.* **215**: 33 (1996); Bianchi *et al., Brain Res. Bull.* **40**: 269 (1996). Indeed, increases in cerebrospinal fluid and plasma levels of β-endorphins have been shown to modulate and optimize behavioral patterns exhibited in patients suffering from str ss, psychiatric disorders, alcoholism, drug addition, obesity, and diabetes. Ryu *et al., Am. J. Chin. Med.* **24**: 193 (1996); Odagiri *et al., Int. J. Sports Med.* **17**: 325 (1996); Dalayeun *et al., Biomed. Pharmacother.* **47**: 311 (1993); Gianoulakis *et al., J. Psychiatry Neurosci.* **18**: 148 (1993). Thes increases also promote natural killer cell mediated cytotoxicity. Jonsdottir *et al., Regul. Pept.* **23**: 113 1996); Sacerdote *et al., Regul. Pept.* **63**: 79 (1996).

[0006] Analgesia also is affected by binding of a pain mediator called "substance P" to its receptor. There are many similarities between the terminals of opioid neurons and the terminals of substance P sensitive neurons. For example, both types of terminals mediate pain sensation in the spinal cord. Jessel *et al., Nature* **268**: 549 (1977). As indicated, for example, in Japanese patent document JP 3133998, substance P receptors have been shown to act as analgesics by masking the activity of substance P. According to PCT application WO 92/16547, the NK-1 receptor preferentially binds substance P and can be used to treat pain, inflammatory disease, mental illness and stress.

[0007] Patients afflicted with conditions such as stress, psychiatric disorders, alcoholism, drug addition, obesity, and diabetes may obtain some measure of relief from an above-normal level of endogenous opioid peptides in their plasma. Clinical relief of symptoms of these conditions have been associated with the binding of opioid peptides with their receptors, which is directly correlated with the level of opioid peptides in the patient's plasma and cerebrospinal fluid. Patients also may benefit from increased levels of substance P receptors or substance P analogs. See WO 92/16547, *supra*, and PCT application WO 91/02745. To date, no adverse reaction has been associated with physiological increases in plasma or cerebrospinal fluid levels of β-endorphins, enkephalins or substance P receptors.

[0008] The use of drugs to increase the production and/or secretion of opioid peptides may provide temporary relief, but uncontrollable drug metabolism and rough dosage eventually will overtax the "sick" neurons and their counterparts. Furthermore, the side effects of drugs are numerous and undesirable. Opioid peptides themselves and opioid peptide receptors have been administered as sedatives and analgesics, see U.S. patent No. 4,123,523, but the effects of such administrations are short-lived.

[0009] Xenogeneic tumor cells secreting β-endorphin have been transplanted into spinal cord cerebro-spinal fluid space of rats, producing analgesic effects. Saitoh *et al., Cell Trans.* **4** (Supp. 1): S13-7 (1995). The transplanted cells were reported to survive for one month, and *in vitro* studies indicated that the cells would secrete β-endorphin for one month. AtT-20 cells and AtT-20/hENK cells, which secrete β-endorphin and enkephalin, respectively, were implanted into mouse spinal subarachnoid space to investigate their use as a therapy for pain. Wu *et al., J. Neurosci.* **14**(8): 4806 (1994); *J. Neural Transplant. Plast.* **4**(1): 15 (1993). But these procedures are very invasive and therefore very dangerous, since they involve the transplantation of cells directly into cerebrospinal fluid or spinal subarach-

noid space. Also, only a limited number of cells are transplanted, making the amount of opioid peptide provided by these methods limited.

[0010] A need therefore exists for a method of long term analgesia by supplying a peptide that binds to opioid receptors or that interferes with binding of substance P to its receptors *in vivo* over a long time period. Such a method would be useful for treating chronic pain and psychiatric conditions that involve abnormal perception, such as depression, chronic anxiety syndromes, paranoia, alcoholism, and drug addiction, and other diseases in which opioid neurons and substance P terminals play a role.

SUMMARY OF THE INVENTION

[0011] Accordingly, it is an object of the present invention to provide a method of treating psychiatric conditions that involve abnormal perception, such as depression, chronic anxiety syndromes, paranoia, alcoholism, and drug addiction, chronic pain, and other diseases in which opioid neurons and substance P sensitive neurons play a role. It is also an object of the present invention to provide a composition for performing this method.

[0012] In accordance with this and other objects of the invention, there is provided a method of continuously supplying *in vivo* a peptide that can bind to opioid receptors or that can interfere with binding of substance P to its receptor comprising the steps of (a) transducing myogenic cells with DNA encoding the peptide, and (b) administering the transduced myogenic cells to a patient, such that the cells continuously produce the peptide. In one embodiment, the analgesic peptide is selected from the group consisting of an opioid peptide, a polypeptide that binds substance P and a substance P analog. In one embodiment, the myogenic cells are selected from the group consisting of myoblasts, myotubes, and muscle cells. In another embodiment, the cells are transduced with DNA encoding multiple copy sequences of the peptide separated by cleavage sites. In another embodiment, the transduced cells are administered by intramuscular injection into a paraspinal muscle of the patient. In yet another embodiment, large chondroitin-6-sulfate proteoglycan or insulin is administered with the transduced myogenic cells. Co-administration of an immunosuppressant also is preferred in some embodiments.

[0013] The invention also provides a method of continuously supplying *in vivo* a naturally occurring analgesic peptide comprising the steps of (a) transducing myogenic cells with DNA containing a promoter for an endogenous structural gene encoding the peptide, and (b) administering the transduced myogenic cells to a patient, such that the cells continuously produce the peptide.

[0014] The invention further provides a composition for continuously supplying *in vivo* a peptide that binds an opioid receptor or that interferes with binding of substance P to its receptor, comprising the steps of (a) transducing myogenic cells that contain heterologous DNA and that express the peptide, and a pharmaceutically acceptable carrier. In one embodiment, the heterologous DNA comprises a gene encoding the peptide and a promoter. In another embodiment, the heterologous DNA comprises a promoter for an endogenous structural gene encoding the peptide. In another embodiment, the composition additionally comprises large chondroitin-6-sulfate proteoglycan or insulin.

[0015] Additional objects and advantages of the invention are set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0016] It has been discovered that genetically transduced myogenic cells can be employed to provide a long-term, continuous supply of a peptide having analgesic activity. This method is useful for treating chronic pain as well as psychiatric conditions that involve abnormal perception, such as depression, chronic anxiety syndromes, paranoia, alcoholism, and drug addiction, and other diseases in which neurons that bind opioids and/or neurons that bind substance P play a role. Such conditions have not been treated heretofore by long-term administration of analgesic peptide *in vivo*.

[0017] Analgesic peptides suitable for the invention are peptides that bind opioid receptors or that interfere with binding of substance P to its receptor. Among these peptides are opioid peptides, polypeptides that bind substance P, and peptides that are substance P analogs. In this context, the phrase "polypeptide that binds substance P" denotes a peptide or protein that has affinity for substance P such as, for example, substance P receptor protein or a peptide or peptide analog derived from this receptor and that retain the ability to bind substance P. Such peptides and proteins bind substance P and thereby interfere with the binding of substance P with its receptor. The skilled artisan can test binding to substance P with an assay. Substance P analogs act as analgesics by interfering with binding between substance P and its receptor. For example, PCT application WO 91/02745, *supra*, discloses analogs that do not exhibit the natural activity of substance P but that act as competitive inhibitors of substance P.

[0018] In accordance with the invention, myogenic cells are transduced *ex vivo* so that they express at least one of the above-enumerated peptides, either while in cell culture, or after differentiation *in vivo*. Cells that have been transduced with a gene encoding such a peptide are administered to the patient, for example, by injection into muscle or adipos tissue of the patient. The transduced cells can survive and grow in the recipient tissue. For example, cells injected into muscle tis-

sue can form myotubes and mature into muscle fibers. Cells injected int adipose tissu can survive and be converted into fat cells. Transduced cells injected into both types of tissue can express the desired analgesic peptide continuously. The expressed peptide exits the cell and travels through the blood to other areas of the body, including the spinal cord and brain.

[0019] Myoblast transfer therapy (MTT) has been used to treat muscle weakness and degeneration and is a useful technique for administering cells that express an analgesic peptide. See U.S. patent No. 5,130,141, the contents of which are incorporated herein by reference. In accordance with this method, genetically normal myogenic cells are administered to a myopathic muscle of the patient, thereby increasing muscle function, locomotive patterns and respiratory function. Normal myoblast transfer therapy has been shown to produce the missing protein dystrophin for up to six years in Duchenne muscular dystrophy patients. Law *et al., Cell Transplantation* 6: 95-100 (1997).

[0020] Although early myoblast transfer studies used muscle as the recipient tissue, other tissues also can be used. For example, myoblasts can grow after their injection or surgical implantation into adipose tissue, as described by Satoh *et al., Transplantation Proceedings* 24: 3017-19 (1992).

[0021] Myoblasts have been transduced with genes for Factor IX, erythropoietin (EPO), and human growth hormone, and the Fas ligand to increase circulating levels of these proteins. Thompson, *Thromb. and Haemost.* 74(1): 45 (1995); Hamamori *et al., J. Clin. Invest.* 95: 1808 (1995), and *Human Gene Therapy* 5: 1349 (1994); Barr *et al., Science* 254: 1507 (1991); Dhawan *et al., Science* 254: 1509 (1991); Lau *et al., Science* 273: 109 (1996). The success of these methods has varied. According to Thompson (1995), for instance, preliminary data suggest that human myoblasts removed from the body survived less well in culture and progressively lost their ability to express factor IX. Lau *et al.* (1996), reports that expression of the Fas ligand was local and appeared to cease after 80 days. On the other hand, Hamamori *et al.* (1994) reports that the *in vivo* implantation of a stable, high level, EPO-producing muscle cell clone resulted in sustained high serum EPO levels for three months, and Dhawan *et al.* (1991) states that transduced myoblasts continued to secrete hGH after they differentiated into myotubes, with no difference in secretion levels between myoblasts and myotubes.

[0022] Transduced myoblasts have not been used previously to supply an analgesic peptide continuously *in vivo*, however. Furthermore, although gene therapy has been studied as a means of supplying opioid peptides *in vivo*, the transduced cells were injected directly into spinal cord, cerebro-spinal fluid or spinal subarachnoid space. Saitoh *et al., Cell Trans.* 4 (Supp. 1): S13-7 (1995); Wu *et al., J. Neurosci.*, 14(8): 4806 (1994); Wu *et al., J. Neural Transplant. Plast.* 4(1): 15 (1993). As discussed above, these methods are very invasive, only a limited number of cells are transplanted, and the transduced cells expressed the opioid peptides for only one month. In accordance with the present invention, by contrast, the transduced myoblasts are not injected into the central nervous system. Moreover, unlike the short-term expression of opioid peptides effected in conventional gene therapies, the present invention provides a continuous, long-term supply of opioid peptides which lasts, for example, up to at least six years. These aspects of the present invention represent distinct advantages that have not been appreciated.

[0023] Myogenic cells that are suitable for the present invention include myoblasts, myotubes, and muscle fiber cells. Myoblasts are particularly preferred, in accordance with one embodiment of the invention. Myoblasts are mononuclear embryonic muscle cells that differentiate into multinucleated myotubes. Each nucleus of a myoblast contains over 100,000 genes, including genes for opioid peptides such as β-endorphins and enkephalins. Myoblasts divide extensively, migrate, fuse naturally to form syncytia, lose MHC-1 antigens soon after fusion, and constitute about 50% of the dry body weight of humans. Myoblasts are unusual in that they are capable of natural cell fusion among themselves and with mature muscle fibers. As a result of this fusion, a transduced myoblast transfers its nucleus and therefore all of its genes to the cell with which it fuses, which may be a genetically normal or an abnormal muscle cell.

[0024] Myogenic cells can be obtained from a patient to be treated, from a relative, or from another human or other animal donor. In a typical procedure, 1 to 2 grams of skeletal muscle is harvested from a donor. Myogenic cells also can be cultured or produced by cloning methods known to those skilled in the art as shown, for example, in U.S. patent No. 5,130,141.

[0025] In accordance with one embodiment of the invention, muscle cells from a human or animal donor are stimulated 0-3 days before harvesting to produce a reservoir of satellite cells that are myoblast reserves in mature muscles. The myogenic cells can by stimulated by, for example, injuring the cells with a number of needle probings, or by sonication.

[0026] In accordance with one embodiment of the invention, harvested cells are processed to obtain a pure culture of myoblasts. See Law *et al. Cell Transplant.* 1: 235 (1992); *Cell Transplant.* 2: 485 (1993); *Muscle and Nerve* 11: 525-33 (1988). For example, a muscle biopsy is dissociated with 0.1% collagenase and 0.2% crude trypsin in phosphate buffered saline at pH 73. The mixture is stirred for 45 minutes, with three changes of enzyme solution alternated with three changes of a neutralizing medium comprising 100 parts Dulbecco's modified Eagle's Medium (DMEM, Gibco) containing 0.37% $NaHCO_3$ and 4mM glutamine; 10 parts horse serum and 1% antibiotic-anti-mycotic.

[0027] Pursuant to one embodiment of the present

invention, the harvested myogenic cells are transduced *ex vivo* with DNA encoding a peptide that either binds an endorphin receptor or that inhibits binding of substance P to its receptor. Peptides that are known to have suitable activity in this context are β-endorphin, α-endorphin, gamma-endorphin, delta-endorphin, Met sup 5 (a five amino acid residue peptide with endorphin-like activity), active endorphin peptides comprising parts of the β-endorphin sequence, enkephalin, an NK-1 receptor, a polypeptide that binds substance P, or a substance P analog that competitively inhibits the binding of substance P to its receptor. The phrase "substance P analog" denotes a peptide that comprises the five carboxy-terminal amino acid sequence of substance P (-Phe-Phe-Gly-Leu-Met) and that binds to the substance P receptor, inhibiting substance P activity. See Payan, *Ann. Rev. Med.* **40**: 341 (1989), and PCT application WO 91/02745.

[0028] Additional peptides can be found by kinetics experiments that reveal whether a given peptide either binds to an opioid receptor or competes for binding between substance P and its receptor. These experiments can be done routinely. See, for example, PCT application WO 92/16547.

[0029] DNA sequences useful for the invention are known or can be designed by those skilled in the art from known amino acid sequences of the peptides. For example, Saitoh *et al., Cell Trans.* **4** (Supp. 1) : S13-7 (1995), discloses a DNA sequence that codes for β-endorphin; Wu *et al.* (1993, 1994), *supra*, disclose sequences for β-endorphin and enkephalin; U.S. patent No. 4,123,523 discloses amino acid sequences of β-endorphin peptides; PCT application WO 92/16547 discloses a gene encoding the substance P receptor NK-1; Japanese patent document JP 3133998 discloses the amino acid sequence of a substance P receptor; and PCT application WO 91/02745 discloses the amino acid sequences of several substance P analogs, such as deletion and addition mutants of substance P.

[0030] In accordance with one embodiment of the invention, the DNA encodes a plurality of copies of a peptide that produces analgesia. In a preferred embodiment the peptide is an opioid peptide and regions of the DNA that code for multiple copies are separated by cleavage sites (see PCT application WO 96/17941). This embodiment can provide an amplified amount of a naturally occurring peptide.

[0031] The transduction of myogenic cells with a DNA sequence can be effected via known methods, such as those reported by Thompson (1995) and Hamamori *et al.* (1995), *supra*. Generally, a DNA construct is used that contains a promoter upstream of the structural gene that encodes the desired peptide. Suitable promoters are described, for example, in U.S. patent No. 5,618,698.

[0032] According to another embodiment of the present invention, the harvested myogenic cells are transduced *ex vivo* with a DNA containing a promoter that can link up with and function (*i.e.*, turn on or increase expressi n) with an endogenous gen within the nucleus of a myogenic cell. In this embodiment, DNA comprising a regulatory sequence, an exon and a splice donor are introduced into a cell by homologous recombination into the cell's genome at a preselected site. The introduction of this DNA results in the production of a new transcription unit in which the regulatory sequence, exon and splice donor site are operatively linked to the endogenous gene.

[0033] The introduction of DNA typically is followed by selection of cells that have received a promoter in a desired location, to turn on the desired gene. Applicable selection methodology is described, for instance, in U.S. patent Nos. 5,641,670 and 5,272,071. Selection techniques also are described by Mansour *et al., Nature* 136: 348, 349 (1988). After selection, the cells which express the desired gene are cultured and then introduced into a patient.

[0034] The transduced myogenic cells are cultured to produce a sufficient quantity of cells for administration to the patient by any of a variety of methods known in the art. For example, see Law *et al.* (1988, 1992), *supra*. The amount of cells cultured will depend on the condition of the patient and the severity of the disease being treated. For example, from about 1 billion to about 100 billion myoblasts can be cultured for administration to a patient. In accordance with one embodiment of the invention, cells are cultured in the neutralizing medium described above, supplemented with two parts of chick embryo extract. Cells are fed fresh growth medium every two days, and are incubated in 7% CO_2 at 37°C for 35-40 days.

[0035] In accordance with one embodiment of the invention, the transduced cells are administered to the patient by intramuscular injection. Law *et al., Cell Transplant.* 1: 235 (1992); *loc. cit.* 2: 485 (1993); Law *et al. Exp. Neurol., Transplant Proc.* 29: 2234 (1997). The amount of opioid peptides provided in accordance with the present invention can be controlled by selecting the number of muscles injected and the number of cells injected. In accordance with one embodiment of the invention, the direction of injection is controlled to optimize the number of transduced cells delivered to recipient muscle fibers. For example, injecting the administered cells diagonally through muscle fibers has been shown to maximize the resulting number of muscle fibers fused with administered cells.

[0036] Pursuant to another embodiment of the invention, transduced cells are administered to specific muscles which help target the cells to a location between laminae IV and V of the spinal cord. For example, the transduced cells can be injected into paraspinal muscles or neck muscles, such as the levator scapulae. Although transduced myogenic cells administered anywhere in the body will secrete peptides that will travel through the blood and reach the spinal receptors, targeting the administration of the cells to paraspinal mus-

cles or neck muscles that are in proximity to the spinal cord is expected to result in more peptides reaching th receptors more rapidly, thereby increasing the efficacy of the method.

[0037] The transduced cells also may be administered by surgical implantation into the patient. The cells can be implanted in, for example, adipose tissue.

[0038] In a further embodiment, the patient also is given an effective amount of an immunosuppressant to minimize rejection of the transduced cells. See U.S. patent No. 5,130,141 and Law *et al.* (1992, 1993), *supra*. For example, cyclosporin A, another immunosuppressant, or combinations of immunosuppressants, can be given in accordance with known procedures. Suitable dosage forms, dosage amounts and dosing schedules are known in the art. For example, cyclosporin A can be given orally in a daily dose of about 7 mg/kg body weight. A typical dosing schedule comprises giving the daily dose in two divided doses, and the patient's whole blood can be monitored to maintain a trough level of about 250 mg/ml cyclosporin A.

[0039] In accordance with one embodiment of the invention, fusion of the transduced myoblasts is facilitated by administration of large chondroitin-6-sulfate proteoglycan (LC6SP) as described in the above-cited U.S. application Serial No. 08/477,377. Trauma from injecting myoblasts into the extracellular matrix triggers the release of basic fibroblast growth factor and large chondroitin-6-sulfate proteoglycan. These released molecules stimulate myoblast proliferation. Increasing the level of large chondroitin-6-sulfate proteoglycan at the injection site facilitates myoblast fusion and proliferation. Accordingly, in accordance with one embodiment of the invention, large chondroitin-6-sulfate proteoglycan preferably is administered with the transduced myoblasts.

[0040] In accordance with one embodiment of the invention, the large chondroitin-6-sulfate proteoglycan is under-sulphated. See Hutchison *et al., Devel. Biol.* **115**: 78-83 (1986). Large chondroitin-6-sulfate proteoglycan is believed to be synthesized in an under-sulphated form pre-fusion, but becomes more highly sulphated post-fusion. *Id.* As used here, therefore, the phrase "under-sulphated large chondroitin-6-sulfate proteoglycan" denotes a degree of sulphation that is about the same as that observed in naturally occurring large chondroitin-6-sulfate proteoglycan from cells just before fusion. In accordance with this aspect of the invention under-sulphated large chondroitin-6-sulfate proteoglycan is administered at a concentration between about 5μM to about 5 mM. Chondroitin-6-sulfate can be administered together with the transduced cells, or can be given in a separate formulation as a separate injection.

[0041] Insulin also facilitates proliferation of myoblasts and promotes myotube development. In accordance with one aspect of the invention, therefore, insulin is administered with the transduced myocytes. For example about 0.2mM of insulin can be given, either as part of th same formulation as the cells, or as a separate formulation, given, for example, in a separate injection.

[0042] In accordance with one embodiment of th invention, undesirable effects from over-production of the desired peptide are regulated with agonists such as naloxone or SP-40,40. Pomeranz *et al., Altern. Thor. Health Med.* **2**: 85 (1996); Choi-Miura *et al., Biol. Pharm. Bull.* **16**: 228 (1993); Pomeranz *et al., Exp Neurol.* **54**: 172 (1977). For example, if the endogenous level of the peptide becomes too high, naloxone or SP-40,40 can be administered to counteract the peptide's effects. Typical symptoms of over-production of an analgesic peptide include extreme drowsiness, low respiratory rate, cyanosis, low blood pressure, symmetrical, pinpoint pupils, and depressed urine formation. A usual course of naloxone treatment involves giving small intravenous or intramuscular doses of naloxone (about 0.4 mg to about 0.8 mg). Symptoms frequently improve after the first dose, but can be repeated after 2-3 minutes, up to a total dose of about 10 mg.

[0043] As discussed above, the administration of transduced myogenic cells in accordance with the present invention provides a continuous, long-term supply of an analgesic peptide *in vivo*. The peptide travels from the site of synthesis such as from muscle or adipose tissue and reach sensory nerve endings, the spinal cord and brain, where it combines with nerve cell receptors to produce analgesia. Analgesia produced by the peptide is useful for treating chronic pain and psychiatric conditions that involve abnormal perception, such as depression, chronic anxiety syndromes, paranoia, alcoholism, and drug addiction, and other diseases in which opioid neurons and substance P terminals play a role. The continuous long-term supply of an analgesic peptide *in vivo* as a medical treatment offers a novel methodology of treating these conditions.

[0044] The invention also provides a composition that makes an analgesic peptide that binds to opioid receptors or interferes with binding of substance P to its receptor *in vivo*. In one embodiment, the composition comprises myogenic cells containing heterologous DNA coding for an analgesic peptide together with one or more pharmaceutically acceptable carriers.

[0045] Examples of suitable pharmaceutical carriers include diluents, solvents, buffers, and/or preservatives. An example of a pharmaceutically acceptable carrier is phosphate buffer that contains NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, salts, preservatives, buffers and the like, as described in REMINGTON's PHARMACEUTICAL SCIENCES, 15th Ed. Easton: Mack Publishing Co., pages 1405-1412 and pages 1461-1487 (1975), and THE NATIONAL FORMULARY XIV., 14th Ed. Washington: American Pharmaceutical Association (1975). Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous

carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the binding composition are adjusted according to routine skills in the art. See GOODMAN AND GILMAN's THE PHARMACOLOGICAL BASIS FOR THERAPEUTICS (7th ed.)

[0046] In accordance with one embodiment, the composition comprises transduced myogenic cells, large chondroitin-6-sulfate proteoglycan, and a pharmaceutically acceptable carrier.

[0047] In accordance with another embodiment, the composition comprises transduced myogenic cells, insulin, and a pharmaceutically acceptable carrier.

[0048] The embodiments of the invention are further illustrated through the following examples which show aspects of the invention in detail. These examples illustrate specific elements of the invention and are not to be construed as limiting the scope thereof.

Example 1. Treatment of Patient Suffering from Depression by Injection into Muscle Tissue

[0049] The skeletal muscles of a patient suffering from a psychiatric condition involving depression are stimulated by numerous needle probings to produce a reservoir of satellite myoblast cells. Three days later, the patient is placed under general anesthesia, and 2 g of skeletal muscle are harvested from the patient. The harvested muscle is processed to obtain a pure culture of myoblasts. The harvested muscle is dissected free of skin and other tissue and the cells are dissociated with 0.1% collagenase and 0.2% crude trypsin in phosphate buffered saline at pH 7.3. The mixture is stirred for 45 minutes, with three changes of enzyme solution alternated with three changes of a neutralizing medium comprising 100 parts Dulbecco's modified Eagle's Medium (DMEM, Gibco) containing 0.37% $NaHCO_3$ and 4mM glutamine; 10 parts horse serum and 1% antibiotic-antimycotic.

[0050] These myoblasts are transduced with DNA containing a gene for enkephalin and a suitable promoter. The transduced myoblasts then are cultured in the neutralizing medium described above supplemented with 2 parts of chick embryo extract. The cells are fed fresh growth medium every 2 days, and are incubated in 7% CO_2 at 37°C for 40 days, when about 2 billion myoblast cells (progeny of the transduced myogenic cells) are present.

[0051] The patient again is placed under general anesthesia and the progeny of the transduced myogenic cells are injected intramuscularly, into paraspinal muscles of the patient. Within one week thereafter, the patient's symptoms should begin to ameliorate.

Example 2. Treatment of Patient Suffering from Depression by Injection into Adipose Tissue

[0052] Myoblasts are obtained and transduced as described in Example 1 to form about 10 billion progeny myoblast cells. The patient's breast tissue is anesthetized and the cells are injected into the anesthetized tissue. Within one week thereafter the patient's symptoms should begin to ameliorate.

Example 3. Treatment of Patient Suffering from Alcoholism

[0053] The skeletal muscles of a patient suffering from alcoholism are stimulated by sonication to produce a reservoir of satellite myoblast cells. Three days later, the patient is placed under general anesthesia, and 2 g of skeletal muscle are harvested from the patient. The harvested muscle is processed to obtain a pure culture of myoblasts as described in Example 1 above.

[0054] These myoblasts are transduced with DNA containing a promoter for an endogenous β-endorphin gene. The transduced myoblasts then are cultured as described in Example 1 above, until 50 billion cells are obtained.

[0055] The patient is again placed under general anesthesia and the progeny of the transduced myogenic cells are injected into paraspinal muscles of the patient.

[0056] Within one week after the procedure, the patient's symptoms begin to be relieved.

Example 4. Composition For Providing a Long-Term, Continuous Supply of Enkephalin In Vivo

[0057] The following composition is provided:

> 1 billion myogenic cells transduced with DNA that codes for enkephalin; and a phosphate buffer containing NaCl and human serum albumen as a pharmaceutically acceptable carrier.

Example 5. Composition For Providing a Long-Term, Continuous Supply of β-Endorphin In Vivo

[0058] The following composition is provided:

> 1 billion myogenic cells transduced with DNA that codes for a promoter of a human endorphin gene; and water as a pharmaceutically acceptable carrier.

[0059] It will be apparent to those skilled in the art that various modifications and variations can be made to the processes and compositions of this invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

Claims

1. A composition for supplying a peptide in vivo that binds to an opioid receptor or that interferes with binding of substance P to its receptor, comprising (i) myogenic cells that contain heterologous DNA encoding the peptide, such that the myogenic cells express the peptide, and (ii) a pharmaceutically acceptable carrier.

2. The composition according to claim 1, wherein the heterologous DNA comprises a structural gene for the peptide and a promoter.

3. The composition of claim 2, wherein the heterologous DNA comprises multiple copies of a gene for the peptide.

4. The composition of claim 1, wherein the peptide is an opioid peptide.

5. The composition of claim 4, wherein the opioid peptide is selected from the group consisting of β-endorphin, α-endorphin, gamma-endorphin, delta-endorphin, and Met sup 5.

6. The composition of claim 4, wherein the opioid peptide is selected from the group consisting of β-endorphins and enkephalins, and wherein the heterologous DNA comprises a promoter for an endogenous structural gene encoding the peptide.

7. The composition of claim 1, wherein the peptide is a polypeptide that binds substance P.

8. The composition of claim 1, wherein the peptide is a substance P analog comprising the sequence - Phe-Phe-Gly-Leu-Met.

9. The composition of claim 1, further comprising large chondroitin-6-sulfate proteoglycan.

10. The composition of claim 9, wherein the large chondroitin-6-sulfate proteoglycan is under-sulphated.

11. The composition of claim 1, further comprising insulin.

12. The use of myogenic cells that contain heterologous DNA encoding a peptide that binds an opioid receptor or that interferes with binding of substance P to its receptor for therapy.

13. The use of myogenic cells that contain heterologous DNA encoding a peptide that binds an opioid receptor or that interferes with binding of substance P to its receptor for the manufacture of a pharmaceutical composition for supplying *in vivo* said peptide.

14. The use of claim 13, wherein the peptide is selected from the group consisting of an opioid peptide, a polypeptide that binds substance P, and a substance P analog.

15. The use of claim 13, wherein the myogenic cells are selected from the group consisting of myoblasts, myotubes, and muscle fiber cells.

16. The use of claim 13, wherein the myogenic cells are harvested from skeletal muscle tissue of the patient.

17. The use of claim 13, wherein the myogenic cells are harvested from skeletal muscle tissue of a normal donor.

18. The use of claim 16, wherein the skeletal tissue is stimulated before harvesting to produce a reservoir of satellite myoblast cells.

19. The use of claim 16, wherein the harvested myogenic cells are processed to produce a purified sample of myoblast cells.

20. The use of claim 13, wherein the transduced myogenic cells are cultured to produce a sample of transduced myogenic cell progeny comprising at least 1 billion cells.

21. The use of claim 13, wherein the peptide is an opioid peptide.

22. The use of claim 21, wherein the opioid peptide is selected from the group consisting of β-endorphin, α-endorphin, gamma-endorphin, delta-endorphin, Met sup 5, and enkephalin.

23. The use of claim 13, wherein the peptide is a polypeptide that binds substance P.

24. The use of claim 13, wherein the peptide comprises the sequence Phe-Phe-Gly-Leu-Met.

25. The use of claim 13, wherein the DNA comprises two nucleotide sequences, each coding for the peptide, and a segment separating the two nucleotide sequences, wherein the segment codes for a cleavage site.

26. The use of claim 13, wherein the composition is presented in a form adapted to be administered by intramuscular injection.

27. The use of claim 26, wherein the composition is adapted to be injected into a paraspinal muscle of

the patient.

28. The use of claim 26, wherein the composition is adapted to be injected into a levator scapulae muscle of the patient.

29. The use of claim 26, wherein the composition is adapted to be injected into a neck muscle of the patient.

30. The use of claim 13, wherein the composition is administered with large chondroitin-6-sulfate proteoglycan.

31. The use of claim 13, wherein the composition is administered with insulin.

32. The use of claim 13, wherein an immunosuppressant is administered prior to, simultaneously with or subsequent to the administration of the composition.

33. Use of myogenic cells for the manufacture of a composition for continuously supplying an opioid receptor-binding peptide *in vivo*, comprising the steps of

(a) transducing a plurality of myogenic cells, at least some of which contain (i) a gene that codes for the peptide and (ii) a flanking region associated with the gene under conditions conducive to homologous recombination, with DNA that comprises a promoter and a segment that is homologous to the flanking region;

(b) selecting among the plurality for myogenic cells wherein the promoter and the gene are functionally linked;

(c) multiplying the myogenic cells selected in step (b) to produce progeny cells; and

(d) formulating the progeny cells, which continuously produce the peptide, as a composition.

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which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 20 1068

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	TAKEUCHI ET AL: "PRODUCTION OF A THERAPEUTIC PEPTIDE ENKEPHALIN FROM A VARIETY OF NON-ENDOCRINE CELL LINES USING A NOVEL EXPRESSION VECTOR FOR FUSION PEPTIDES" GENE THERAPY, vol. 2, 1995, page 689 XP002084065	1-6, 12-22, 25-29, 32,33	A61K35/34 A61K48/00
Y	see abstract 066	7-11,23, 24,30,31	
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TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C12N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

Although claim 12 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Place of search	Date of completion of the search	Examiner
THE HAGUE	11 November 1998	Sitch, W

CATEGORY OF CITED DOCUMENTS

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Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
Y	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT 79213319, SANDRA ET AL: "REVERSAL BY INSULIN OF CONCANAVALIN A INHIBITION OF MYOTUBE FORMATION AND EVIDENCE FOR COMMON BINDING SITES" XP002084067 * abstract * & ENDOCRINOLOGY, vol. 105, no. 2, August 1979, pages 391-401, ---	11,31	
D,A	WU ET AL: "IMPLANTATION OF ATT-20 OR GENETICALLY MODIFIED ATT-20/HENK CELLS IN MOUSE SPINAL CORD INDUCED ANTINOCICEPTION AND OPIOID TOLERANCE" JOURNAL OF NEUROSCIENCE, vol. 14, no. 8, 1994, pages 4806-4814, XP002084066 * page 4806 * * abstract * -----		TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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